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RECENT USSR WORK ON ETIOLOGY AND IMMUNOLOGY OF CANCER

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At present, the virus etiology of many tumors has been established with certainty. These include certain tumors of insects and fish, various types of sarcoma of chickens and ducks, various forms of leukoses of birds, the carcinoma of frog kidneys, the papillomas of many animals such as cattle, dogs, and rabbits, the cancer of the lactic glands of mice, the leukemia of mice, and human warts.

The viruses which cause these diseases have been photographed under an electron microscope. Some of the largest of them, for instance, the virus of the cancer of the lactic glands of mice, can be observed under an optical microscope after the preparation has been stained according to M. Morozov's method. After these viruses have been introduced into the body of a susceptible animal, they produce tumors identical with those from which they have been isolated.

A very important confirmation of the etiological role played by these viruses was furnished by the investigations of A. Timofeyevskiy and S. Benevolenskaya, who have shown that the viruses transform normal cells into tumor cells in tissue cultures. At present, even the most obstinate opponents of the virus theory do not deny the obvious fact that viruses are the direct cause of the development of many types of tumors.

In the course of the investigation of tumors produced by viruses a very curious phenomenon was discovered. It is easy to isolate a virus producing rabbit papilloma from this type of papilloma. However, as soon as the papilloma has been transformed into a cancer (analogous to the transformation into cancer of the papillomas of the throat or of the urinary bladder of humans), the virus can no longer be isolated from the tumor. Nevertheless, serological investigation indicates that the virus is still present in the tumor. Such viruses, which have lost their pathogenicity, are referred to as masked viruses.

On the other hand, it is known that the growth of tumors may be induced by many factors other than viruses, namely, diverse chemical substances which are known as cancerogens, various types of radiant energy, and the action of hormones and of many other substances and inducing effects. Even the repeated injection of such substances as fructose or chicken protein may result in the growth of a tumor. When tumors have been induced in this manner, no viruses can be isolated from them. What is the etiology of these tumors? Do all these substances and physical effects produce malignant neoplasms, as is believed by the proponents of the polyetiological theory of the origin of tumors, or do they function as pathogenic factors which create suitable conditions under which viruses already present in the organism may unfold their action?

One may assume that in tumors induced by cancerogenic effects the virus is present in a masked form and for that reason cannot be detected. But can it be detected prior to the loss of pathogenicity?

The first experimental work on cancer carried out at our laboratory dealt with an investigation of this problem. We induced tumors in mice by injecting cancerogenic substances subcutaneously. Six to eight months later, as soon as a minor hardening was observed under the skin at the site of the injection of the cancerogenic substance, the tissue was excised and disintegrated and an extract from it filtered through Berkefeld filters. The filtrate, which was absolutely free of cells, was injected subcutaneously into mice that had been

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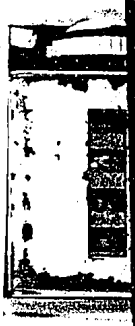
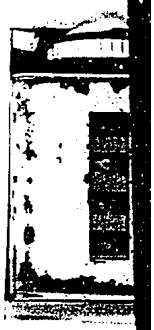


treated prior to that with cancerogenic substances administered in a quantity which does not produce tumors. We assumed that in the initial stage of development of the tumor the virus would not be masked and for that reason would be capable of being detected on the basis of its pathogenic effect. In view of the fact that viruses multiply with the greatest facility in young cells which are in the process of division, and taking into consideration the fact that introduction of cancerogenic substances creates foci of tissue proliferation, we took the precaution of introducing extracts of rudimentary tumors to mice which had been treated prior to that with cancerogenic substances in minimal doses that do not produce tumors.

Our experiments yielded positive results. In approximately 15% of the cases, the cell-free filtrates of sarcomas in the initial state of development induced the appearance of similar tumors. In some cases these tumors could be transmitted by the same method to other mice. Similar results were obtained in analogous experiments on rabbits. These experiments furnished an experimental basis for the hypothesis that viruses play a role in the etiology of tumors that have been induced by cancerogenic substances. However, our experiments could be reproduced only with difficulty. They gave positive results in a relatively small number of cases. The attempts to detect viruses in an analogous manner in the tumors of other animals [besides rabbits and mice] that have been induced by cancerogenic substances were unsuccessful. Under the circumstances we chose other ways of solving the problem. At present, it is clear that we committed an error in discontinuing our investigations. Timofeyevskiy and Benevolenskaya have recently reported that the cell-free extract of rat sarcoma produced malignization of rat fibroblasts in a tissue culture exposed to the parallel action of a cancerogenic substance which does not produce malignization alone. In these experiments the same principle of the combined action of a virus and of a cancerogenic substance was applied that had been applied in our investigation. Here a process has been reproduced in vitro which we observed in vivo in our experiments. These data and other results indicate the necessity of resuming our previous experiments and of investigating the role of viruses in the generation of induced tumors by applying virological methods of investigation.

One of the principal arguments advanced by the opponents of the virus theory of the generation of tumors was the fact that there are no foreign proteins in tumors which are not transmitted by filtrates or in human tumors. However, a virus, whether masked or not masked, consists of protein which is foreign to the organism. If heterogenous protein is absent in tumors, there can be no immunity to tumors, because immunity is the reaction of the organism to heterogenous substances. The problem of the presence or absence of heterogenous antigens in tumors is for that reason a cardinal problem, the solution of which is essential not only for proving the virus theory of the origin of tumors but also for establishing whether immunological processes in cancer are possible or not. The reason why many investigators were unable to find heterogenous proteins possessing antigenic characteristics in tumors is as follows.

One may assume that the failure of some of these attempts was due to the low concentration of the antigen in the tumors and to the low sensitivity of the reactions used for the detection of these antigens. For that reason, we decided to investigate not merely the extracts of tumor tissue, as has been done by many investigators, but protein factions isolated from these extracts. In doing this, we assumed that the antigen for which we searched will be contained in one of the fractions in a more concentrated form. Originally we searched for the antigen by using the reaction of complement fixation carried out at low temperatures. Subsequently, we developed the highly sensitive reaction of anaphylaxis accompanied by desensitization, which made it possible



to differentiate clearly between tumor proteins and the proteins of normal tissues. By using this reaction in work carried out together with N. Nartsissov, Z. Baydakova, and others, we succeeded in demonstrating that in tumors of animals induced by cancerogenic substances there are antigens which are absent in the tissues of healthy animals and which produce the formation of antibodies during the process of the development of tumors. Subsequently, our collaborators A. Gardash'yan, D. Levina, V. Gorodilova, L. Shershul'skaya, and others, isolated specific antigens from all strains of transmissible tumors which were available to us, as well as from all investigated human tumors.

One may state on the basis of this that tumors contain specific antigens. The principal objection to the virus theory of the origin of tumors has thus been eliminated and the study of the immunology of tumors has been given a sound theoretical basis for the first time.

After the solution of this problem many other problems arose. Do the specific antigens of tumors consist of the substance of viruses or perhaps of the modified protein of normal tissues? Is there only one specific antigen in tumors or are there several? In what part of the cell are specific antigens located? Do the specific antigens induce the formation of antibodies and in this manner create immunity? These and many other problems which were equally complex had to be solved by us. The persistent work of a large group of investigators was necessary in order to supply answers to some of the questions involved.

Z. Baydakova, R. Radzikhovskaya, A. Vadova, and Z. Postnikova proved that it is possible to induce a high degree of immunity to tumor viruses. However, the animals which were immunized to these viruses (those of chicken sarcoma and rabbit papilloma) got sick in 100 percent of cases after the corresponding tumor tissue was administered to them. It has been known for a long time that immunity to tumor tissue arises during the natural resorption of tumors. However, nobody had investigated this phenomenon on tumors the virus etiology of which was established with certainty. The observations of R. Radzikhovskaya have shown that chickens with resorbed tumors were in the majority of cases immune both to the virus and to the tumor tissue.

Thus, immunization with the virus creats immunity to the virus and does not produce immunity to transplanted tumor cells, while immunity to the resorbed tumor creates immunity both to the virus and to tumor cells. One can draw only one conclusion from these experiments, namely, that the tumor cells contain in addition to the virus another heterogenous protein which brings about immunization to tumor tissue. How can this be proven by direct experiment?

To obtain this proof, it was necessary to eliminate the virus from tumor tissue and to investigate the antigenic composition of the proteins remaining in this tissue. A. Vadova, Z. Postnikova, Ye. Barabadze, and V. Artamonova had demonstrated that the papilloma virus is adsorbed on erythrocytes and that by repeated adsorption one may completely free the tissue of the virus component. When Artamonova investigated the proteins of the tumor tissue which remained after elimination of the virus by this method, she found that they contained an antigen which is absent in normal tissue. Thus, it has been shown for the first time that tissue protein which is distinct from the protein of normal tissues is present in tumors induced by viruses. When the protein of a rabbit carcinoma which had originated from a papilloma and was isolated from the fraction analogous to serum alpha-globulin and beta-globulin and was mixed with papilloma virus in a test tube, the papilloma virus lost its pathogenicity in 30 to 60 minutes. In other words, the virus was masked. On the basis of this, one may conclude that the phenomenon of the masking of tumor viruses which has been investigated for 20 years, but has not been explained hitherto, consists in the blocking of the virus by the modified cell protein which develops in the organism as a result of changes in the synthesis of tissue proteins produced by the multiplication of the virus. Further investigations are necessary in order to establish whether the process of blocking takes place in tumors induced by cancerogenic substances.

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Considerable difficulties were met in connection with the development of methods for establishing the location of the specific antigen in the cell. Without solving this question one could not find an effective method for the concentration and purification of tumor antigens. In connection with this problem, extensive work was carried out by the Institute of Epidemiology and Microbiology imeni N. F. Gamaleya in collaboration with machine construction engineers on the construction of new laboratory equipment. Particularly active in this work was V. Loskutov, Chief of the Technical Administration, Ministry of Machine and Instrument Building USSR. Using the new Soviet rapid separators, it was possible to obtain from the same batch of tumor tissue a sufficient quantity of nuclei, mitochondria, microsomes, and soluble proteins. G. Abelev developed a method for the separation of tumor tissue into all of these components. These components were then investigated by N. Nartsissov in serological experiments.

The most important result of these investigations was the finding that the specific serological activity of the tumor cells is associated with the mitochondria and the first globulin fraction of proteins obtained on saturation of the tumor extract with ammonium sulfate to the extent of 33 percent. These results, obtained in the investigation of experimental tumors and human tumors, create a new basis for work on the problem of the serological diagnosis of cancer.

The extensive experience accumulated by our laboratory in the investigation of the immunology of tumors has made it possible for us to carry out a profitable investigation of the problem of specific prophylaxis of tumors. Hitherto one could not achieve artificial immunization against tumors transmitted by means of material which does not contain living tumor cells, although many attempts were made to establish such immunity. In work carried out with Z. Baydakova and R. Radzikhovskaya, we developed methods whereby we can obtain from a tumor a mixture of antigens that creates a rather high degree of immunity. In the procedure in question a suspension of tumor cells is dissolved by a cancerolytic serum under definite conditions. The cells which remain undissolved or have been only partly dissolved are removed from the lysate by centrifuging.

By using formalinized lysates of this type, in which the serum stabilizes the very labile immunizing proteins, one may create immunity to subsequent implantation of tumor tissue in a considerable percentage of cases (up to 80% of cases in such a severe and metastasizing tumor as the Brown-Pierce carcinoma). By using such vaccines it will perhaps be possible to prevent the recurrence of cancer in patients who have been operated surgically. Clinical observations which are being conducted at present at Moscow and Leningrad will make it possible to answer this question within several years.

These are the fundamental lines along which we conduct our work in order to solve the principal problems of the etiology, pathogenesis, and immunology of tumors. Extensive work on cancer is also being conducted at other Soviet laboratories.

Many questions still remain to be solved. However, we are convinced that Soviet theoretical oncology is on the right path. Under the circumstances we wish to express the hope that our efforts will soon liberate from cancer the Soviet people and the whole of humanity.

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